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Collaborative Feasibility Study of a Biphasic System (Roche Septi-Chek AFB) for Rapid Detection and Isolation of Mycobacteria

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A study to delineate the feasibility of a biphasic-culture approach for detection and isolation of mycobacteria from clinical specimens except blood was conducted in four medical centers. The biphasic system (Septi-Chek AFB, Roche Diagnostic Systems, Nutley, N.J.) was compared with conventional mycobacterial isolation media and the BACTEC system. Septi-Chek AFB showed the highest degree of mycobacterial recovery. In addition, Septi-Chek AFB consistently shortened the time required for recovery of mycobacteria from clinical specimens and supported the growth of small inoculum numbers of stock cultures of 14 mycobacterial species. The study indicates the feasibility and potential advantages of the biphasic approach for detection and isolation of mycobacteria.

The resurgence of tuberculosis (3) and the increase in other mycobacterioses, especially those caused by the *Mycobacterium avium-M. intracellulare* complex, reemphasize the analytical problems that attend the laboratory detection, isolation, and identification of mycobacteria. Application of molecular and/or immunological tools to ease the recognition of *Mycobacterium* spp. directly in a clinical specimen has remained a promise unrealized. The labor-intensive sequences of specimen preparation and medium inoculation under strict containment conditions and prolonged incubation in an atmosphere of 5 to 10% CO_2 must still be performed in most clinical microbiology laboratories. The BACTEC 460 TB system (B-D Diagnostic Instrument Systems, Cockeysville, Md.) addresses part of this problem by reducing the time required for detection of mycobacteria (1). However, this approach is not suitable for all laboratories and does not provide the isolated colonies required for definitive identification and antimicrobial susceptibility tests. The requirements for handling and processing of clinical specimens for isolation of mycobacteria have caused an appreciable number of laboratories to refer such specimens to reference laboratories. The Septi-Chek AFB system (marketed as MB Septi-Chek in Europe by F. Hoffmann-La Roche, Basel, Switzerland) contains biphasic media and a self-contained CO_2 environment. This approach has the potential to expedite processing, obviate carbon dioxide incubation requirements, and facilitate and hasten detection of positive cultures. This report summarizes a collaborative feasibility study of this method in comparison with conventional detection-isolation approaches and the BACTEC instrument.

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MATERIALS AND METHODS

Participants. The participating institutions represent a cross-section of medical facilities. Barnes Hospital (BH) and Long Island Jewish Medical Center (LIJ) are tertiary-care institutions that service urban and suburban communities. The Catholic Medical Center of Brooklyn and Queens, Inc. (CMC), consists of four hospitals and two skilled nursing facilities, several of which serve patients in the inner city. The National Jewish Center for Immunology and Respiratory Medicine (NJ) mycobacteriology service is a reference center. Specimens submitted to NJ are mostly from patients with known mycobacterial infections.

Specimens. The specimens processed for isolation of mycobacteria included sputa, bronchial washings, tracheal aspirates, urine, stool, tissue, and normally sterile body fluids, excluding blood.

Specimen processing. Specimens were processed by standard recommendation (4). Sputa, bronchial washings, and tracheal aspirates were liquified, decontaminated for 15 min with *N*-acetylcysteine and NaOH (final concentration, 2%), and centrifuged for 15 min at 2,000 × *g*, and the sediment was suspended in bovine albumin fraction V (BAF) for a final volume of 2.5 ml. Smears were prepared for acid-fast staining.

Normally sterile body fluids, except cerebrospinal fluid, were centrifuged as described above, and the sediment was suspended in 5.0 ml of BAF. A sample of the BAF was set aside after inoculation of the various cultures to ensure specimen availability in the event of contamination. When contamination was noted, the sample was decontaminated as described above and recultured. Since cerebrospinal fluid is

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rarely contaminated, the sediment was suspended in 2.5 ml of BAF and served as the inoculum for the media.

Urine specimens (first morning urines) were treated for 15 min with an equal volume of 4% NaOH and centrifuged at 3,500 × *g* for 15 min, and the sediments were suspended in a final volume of 2.5 ml of BAF.

Small portions of stool specimens were treated in the same way as sputum.

Tissues and biopsies were homogenized with tissue grinders in sterile 0.9% NaCl, diluted to a final volume of 5.0 ml with BAF, and processed in the same way as normally sterile fluids. Acid-fast staining was performed on all specimens except urine and stools.

Septi-Chek AFB system. The Septi-Chek AFB system consists of a capped bottle containing 30.0 ml of Middlebrook 7H9 broth under enhanced (5 to 8%) CO_2, a paddle with agar media enclosed in a plastic tube, and enrichment broth containing glucose, glycerin, oleic acid, pyridoxal, catalase, albumin, polyoxyethylene 40 stearate, azlocillin, nalidixic acid, trimethoprim, polymyxin B, and amphotericin B. One side of the paddle is covered with nonselective Middlebrook 7H11 agar. The reverse side is divided into two sections: one contains Middlebrook 7H11 agar with nitroacetylaminohydroxypropiopherone for differentiation of *M. tuberculosis* from other mycobacteria; the other section contains chocolate agar for detection of contaminants. The system is processed by removing the cap from the bottle and adding 1 ml of supplement. The supplemented broth is then inoculated with a prescribed amount of the processed specimen. A screw cap is removed from the bottom of the paddle, which is secured to the bottle. The media are inoculated by inverting the assembly, permitting the broth to wash over the agar surfaces. The system was incubated at 35 to 37°C in the upright position. Inspection for growth was performed four times per week for the first 2 weeks, at which times the system was inverted for reinoculation of the agar media. Weekly inspections were performed for an additional 6 weeks without inversion of the system.

Conventional and BACTEC systems. The procedures, followed at all study sites, consisted of inoculation of Lowenstein-Jensen slants (LJ) with 0.25 ml, 7H11 nonselective agar plates with 0.5 ml and, the Septi-Chek AFB with 0.5 ml of the BAF preparation. BH and NJ inoculated BACTEC bottles with 0.5 ml of the BAF suspension. The LJ and 7H11 media were incubated at 35 to 37°C in 5 to 10% CO_2 for 8 weeks. They were inspected for growth concomitantly with the Septi-Chek AFB system. BACTEC was processed as recommended by the manufacturer. Inspections were performed three times per week for the first 3 weeks and then weekly for 5 weeks.

Studies with stock cultures. The following species of mycobacteria were used to establish the ability of the Septi-Chek AFB system to detect mycobacterial species in low numbers: *M. tuberculosis* (two control strains), *M. bovis*, *M. kansasii*, *M. szulgai*, *M. gordonae*, *M. flavescens*, *M. avium* (two strains), *M. intracellulare*, *M. terrae*, *M. fortuitum*, and *M. chelonae*. Since the opacity of the McFarland standards did not reflect the number of viable mycobacteria, 5-ml samples of Septi-Chek AFB broth in sterile culture tubes were inoculated with 0.1 ml of 0.9% NaCl slurries of the bacteria at a McFarland no. 1 density and incubated at 35°C until they reached a 0.5 McFarland density, determined nephelometrically (API nephelometer; Analytab Products, Hicksville, N.Y.). Dilutions of 10^{-4}, 10^{-6}, and 10^{-8} were each inoculated in 0.5- and 1.0-ml volumes into the Septi-Chek AFB system. Colony counts of the dilutions were performed with 7H11 agar.

TABLE 1. Numbers of specimens and mycobacterial isolates by institution

Institution	No. of specimens	No. (%) of mycobacteria
LIJ	424	22 (5.1)
CMC	855	88 (10.2)
BH	2,359	84 (3.6)
NJ	135	118 (87.4)

Controls. *M. tuberculosis* (a multiply resistant strain and an antimicrobial agent-susceptible strain) and *M. avium*, provided by NJ, served as controls for all of the media.

Statistical analysis. Isolation rates were evaluated by the McNemar modification of the χ^2 test (2).

RESULTS

Table 1 summarizes the number of specimens examined and the mycobacteria isolated. As indicated previously, the specimens received at NJ were mostly from patients known to have mycobacterioses. This special function of NJ is reflected in the high percentage of positive recoveries of mycobacteria from a comparatively small number of specimens.

For calculation of recovery rates, we determined the total number of mycobacterial isolates obtained with the Septi-Chek AFB and the comparative medium or system compared with the recovery of each one by itself.

Table 2 summarizes the comparative recoveries of mycobacteria in the various media and systems at the individual study sites. Positive results in the Septi-Chek AFB system were indicated by growth in broth and on nonselective 7H11 agar. The results show that the Septi-Chek AFB system improved the recovery of mycobacteria over the other approaches. At LIJ, 89% of the mycobacteria were isolated in the Septi-Chek AFB system, compared with 63% in LJ ($P = 0.069$) and 67% ($P = 0.132$) in 7H11. The recovery rate of mycobacteria in the Septi-Chek system at CMC was over 93% versus 75% ($P = 0.002$) in LJ and 69% ($P = 0.0001$) for 7H11. At BH, Septi-Chek yielded 85% of the mycobacteria, while 55% ($P = 0.001$) were obtained in LJ, 45% ($P < 0.0001$) were obtained in 7H11, and 76% ($P = 0.244$) were obtained in the BACTEC system. At NJ, Septi-Chek recovered 98% of the mycobacteria, LJ recovered 84% ($P = 0.0003$), 7H11 recovered 90% ($P = 0.013$), and BACTEC recovered 95% ($P = 0.281$).

Table 3 presents the frequencies with which the various species were isolated on each of the media. The improved recovery of mycobacteria in Septi-Chek AFB was evident for all of the species. Overall, 94.2% of the mycobacteria

TABLE 2. Comparison of mycobacterial recoveries

Study site	Total no. of mycobacteria recovered by all systems	% of isolates recovered in:			
		LJ	7H11	BACTEC	Septi-Chek AFB
LIJ	22	63	67		89
CMC	88	75	69		93
BH	84	55	45	76	85
NJ	118	84	90	95	98

TABLE 3. Isolation of mycobacteria in LJ, 7H11, and Septi-Chek AFB

Mycobacterium (no. of isolates)	% of isolates recovered (*P* value)[a] in:		
	LJ	7H11	Septi-Chek
M. avium complex (163)	72.3 (<0.0001)	78.9 (<0.0001)	98.7
M. tuberculosis (108)	83.3 (0.022)	88.3 (0.241)	93.5
M. kansasii (14)	57.1	42.8	100.0
M. chelonae (11)	45.4	54.5	90.9
Other *Mycobacterium* spp. (16)[b]	43.7	43.7	81.2
Total (312)	72.1 (<0.0001)	77.8 (<0.0001)	94.2

[a] The numbers in parentheses are *P* values for the comparison of the conventional media with Septi-Chek AFB. *P* values for the other mycobacteria were not determined because of the paucity of isolates.
[b] The other *Mycobacterium* spp. included six *M. fortuitum* isolates, six *M. gordonae* isolates, two *M. marinum* isolates, one *M. xenopi* isolate, and one *M. bovis* isolate.

were isolated in the Septi-Chek AFB system while 72.1% were recovered from LJ and 77.8% were recovered from 7H11.

Table 4 compares the recovery rates of the BACTEC and Septi-Chek AFB systems. Together, both recovered 195 mycobacteria. Septi-Chek AFB yielded 186 (95.3%), compared with 177 (90.7%) with the BACTEC system. This difference was not statistically significant. The effect of doubling the inoculum volume in the Septi-Chek AFB system to 1.0 ml is shown in Table 5. The larger volume increased the recovery rate from 94.5 to 99.0%, which is not statistically significant.

Table 6 summarizes the average numbers of days required for detection, i.e., the presence of growth in a liquid milieu (BACTEC), or isolation, i.e., colony formation on a solid medium, of the *M. avium* and *M. tuberculosis* complexes. The shortest time required for detection but not isolation of these two mycobacterial complexes was achieved by the BACTEC system. Septi-Chek AFB consistently shortened the time required for isolation of these organisms compared with conventional media. For the *M. avium* complex, Septi-Chek AFB required 19.3 days compared with 28.3 days for LJ and 28.0 days for 7H11. The *M. tuberculosis* complex required 21.8 days for isolation in Septi-Chek AFB versus 25.2 days for LJ and 24.0 days for 7H11. Use of the 1.0-ml inoculum reduced the recovery time for *M. avium* complex by 1.5 days and that for *M. tuberculosis* complex by 2 days compared with the 0.5-ml inoculum.

TABLE 4. Recovery of mycobacteria in BACTEC and Septi-Chek AFB[a]

Mycobacterium (no. of isolates)	% of isolates recovered in:	
	BACTEC	Septi-Chek
M. avium complex (118)	94.9	95.7
M. tuberculosis (45)	93.3	95.5
M. kansasii (10)	80.0	100.0
M. chelonae (11)	54.5	90.9
Other *Mycobacterium* spp. (11)[b]	81.8	90.9
Total (i95)	90.7	95.3

[a] BH and NJ participated in this phase.
[b] The other *Mycobacterium* spp. included four *M. fortuitum* isolates, four *M. gordonae* isolates, one *M. bovis* isolate, and two *M. marinum* isolates.

TABLE 5. Isolation of mycobacteria in Septi-Chek AFB with various inoculum volumes[a]

Mycobacterium (no. of isolates)	% of isolates recovered in:	
	0.5-ml inoculum	1.0-ml inoculum
M. avium complex (49)	91.8	100.0
M. tuberculosis (55)	98.1	98.1
Other *Mycobacterium* spp. (6)[b]	83.3	100.0
Total (110)	94.5	99.0

[a] LIJ and CMC participated in this phase.
[b] The other *Mycobacterium* spp. included four *M. kansasii* isolates, one *M. gordonae* isolate, and one *M. xenopi* isolate.

Contamination of the Septi-Chek AFB system ranged from 4.1 to 5.0%, except in one laboratory with an 8.9% rate. LJ contamination was 2.8%, while 7H11 contamination ranged from 3.2 to 5.2%. The BACTEC instrument displayed 4.7% contamination. Use of a 1.0-ml inoculum did not increase the contamination rate of the Septi-Chek AFB system.

Table 7 summarizes the ability of the Septi-Chek AFB system to detect low numbers of various mycobacterial species. The results of this experiment indicate that only *M. terrae* was difficult to recover in this system at a very low concentration.

The 7H11 medium on the side of the Septi-Chek AFB slide containing two compartments contained nitroacetylaminohydroxypropeopherone. This inhibitor of the *M. tuberculosis* complex as supplied here was not sufficiently selective to differentiate this complex from other mycobacterial species.

DISCUSSION

The resurgence of tuberculosis and the increase of disease due to other mycobacteria, especially the *M. avium* complex, demand that as many clinical microbiology laboratory staffs as possible have the capability to isolate mycobacteria rapidly. The reluctance of many of these laboratory staffs to handle clinical specimens for isolation of mycobacteria is based in part on the need for a dedicated carbon dioxide incubator and storage of several media. This multicenter study indicates that the Septi-Chek AFB system performs as well as, if not better than, the recommended procedures. This system does not require a carbon dioxide incubator, reduces the time required to detect and isolate mycobacteria, and is a compact, easy-to-store assembly.

TABLE 6. Average number of days required for detection or isolation of the *M. avium* and *M. tuberculosis* complexes

Medium (inoculum size [ml])	No. of days to positive culture	
	M. avium	*M. tuberculosis*
Septi-Chek AFB	19.3	21.8
LJ	28.3	25.2
7H11	28.0	24.0
BACTEC[a]	7.0	18.5
Septi-Chek AFB (0.5)[b]	23.5	27.5
Septi-Chek AFB (1.0)[b]	22.0	25.5

[a] Studied at BH and NJC; detection only!
[b] Studied at LIJ and CMC.

TABLE 7. Ability of Septi-Chek AFB to detect small numbers of mycobacteria

Mycobacterium	No. of days to positive culture with Septi-Chek-AFB				No of CFU/ml in inoculum[a]
	0.5-ml inoculum		1.0-ml inoculum		
	Broth	Slides	Broth	Slides	
M. tuberculosis[b]	18	18	18	18	30
M. tuberculosis[c]	18	18	18	18	30
M. bovis	7	7	7	7	30
M. kansasii	4	NG[d]	4	4	10
M. marinum	11	11	11	28	10
M. simiae	14	14	14	14	30
M. scrofulaceum	24	24	24	24	18
M. szulgai	7	7	7	7	10
M. gordonae	7	7	7	7	18
M. flavescens	11	11	28	28	10
M. avium R[b]	4	4	4	4	19
M. avium S[c]	7	7	7	7	22
M. intracellulare	7	7	7	7	30
M. terrae	NG	NG	8	NG	10
M. fortuitum	5	5	5	5	30
M. chelonae	5	5	5	5	16

[a] CFU in 10^{-4}, 10^{-6}, and 10^{-8} dilutions of 0.5 McFarland densities in 5.0 ml of Septi-Chek AFB broth that each served as inocula for the test.
[b] R, antimicrobial agent resistant.
[c] S, completely susceptible to antimicrobial agents.
[d] NG, no growth after 8 weeks of incubation.

No single conventional medium or system recovered all of the mycobacteria isolated in the participants' laboratories. However, the Septi-Chek AFB biphasic approach showed the greatest sensitivity in all of the participating laboratories, accounting for an appreciable increase in the recovery of mycobacteria. This better recovery rate may be attributable to the biphasic nature of the system and the advantage gained from repeated early exposure of the agar media to actively proliferating organisms in the broth phase. The superiority of the Septi-Chek AFB system was demonstrated for all of the mycobacterial species isolated from clinical material. While a slight advantage of the greater volume was demonstrated, the difference was not as great as anticipated. A possible explanation for this is the use of the liquid phase, which permits an increase in an initially small number of mycobacteria in the broth that serves repeatedly as the inoculum for the agar portion of the system.

The slow rate of growth characteristic of most mycobacterial species demands a search for approaches that shorten the time required for mycobacterial detection and isolation. In this study, we used the time required to detect and isolate *M. tuberculosis* and *M. avium* complexes, since these mycobacteria were by far the most frequently encountered representatives. Compared with LJ and 7H11, the Septi-Chek AFB system showed a considerable decrease in the number of days required for isolation of both organisms. While the results obtained with BACTEC, studied at BH and NJ, indicate rapid detection of the mycobacteria, the system does not provide isolated colonies, in contrast to the Septi-Chek AFB and conventional agars. The inoculum volume effect, studied at LIJ and CMC, indicates that the 1.0-ml inoculum decreases the time required for recovery of *M. tuberculosis* and *M. avium* complexes.

The performance of the Septi-Chek AFB system and the ease with which processing, inspection, detection, and isolation can be performed, indicate that the biphasic approach for cultivation of mycobacteria is feasible and practical.

REFERENCES

1. **Heifets, L. B.** 1986. Rapid automated methods (BACTEC system) in clinical mycobacteriology. Semin. Respir. Infect. **1**:242–249.
2. **Ilstrup, D. M.** 1978. Statistical methods employed in the study of blood culture media, p. 31–39. *In* J. A. Washington II (ed.), The detection of septicemia. CRC Press, Inc., West Palm Beach, Fla.
3. **Rieder, H. L., G. M. Cauthen, G. D. Kelly, A. B. Block, and D. E. Snider.** 1989. Tuberculosis in the United States. JAMA **262**:385–389.
4. **Sommers, H. M., and R. C. Good.** 1985. Mycobacterium, p. 216–248. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.